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(54) Title: A METHOD OF REVERSING THYMIC ATROPHY BY ADMINISTERING AN ANTAGONIST OF AN OVERPRODUCED CYTOKINE (57) Abstract The present invention relates, in general, to cytokines and, in particular, to a method of reconstituting the T cell arm of a mammalian immune system in those diseases or conditions in which the T cell pool is damaged. More specifically, the present invention relates to a method of reversing thymic atrophy associated aging and to a method of treating T cell lymphopenia. The methods comprise administering cytokines, agonists thereof, that are underproduced in these physiological states and/or administering antagonists of cytokines that are overproduced.		

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A METHOD OF REVERSING THYMIC ATROPHY BY ADMINISTERING AN ANTAGONIST OF AN OVERPRODUCED CYTOKINE

This application claims priority from Provisional Application No. 60/129,361, filed April 15, 1999, the entire content of which is incorporated by reference.

5 This invention was made, at least in part, with Government support under Grant No. CA28936, awarded by the National Institutes of Health. The Government may have certain rights in the invention.

TECHNICAL FIELD

10 The present invention relates, in general, to cytokines and, in particular, to a method of reconstituting the T cell arm of a mammalian immune system in those diseases or conditions in which the T cell pool is damaged. More specifically, the present
15 invention relates to a method of reversing thymic atrophy associated aging and to a method of treating T cell lymphopenia. The methods comprise administering cytokines, agonists thereof, that are underproduced in these physiological states and/or administering
20 antagonists of cytokines that are overproduced.

BACKGROUND

During the aging of humans, the thymus atrophies, and thymus output of T cells falls (reviewed in Steinmann, Curr. Top. Pathol. 75:43 (1986); Haynes and
25 Hale, Immunological Res. 18:61 (1998)). The thymus is

a chimeric organ comprised of the true thymic epithelial space (TES), which is the site of thymopoiesis, and the perivascular space (PVS) (Steinmann, Curr. Top. Pathol. 75:43 (1986); Tamaoki
5 et al, Keio Med. Jour. 20:57-68 (1975)), which is the site of egress of T cells that have developed in the thymus, and later in life, the site of infiltration of the thymus with peripheral T cells and adipocytes (Haynes, J. Clin. Invest. 103:453-460 (1999); Haynes
10 and Hale, Immunological Res. 18:61 (1998)).

The result of atrophy of the thymus in the normal elderly human is that there may be mild lymphopenia, but otherwise little sequelae of thymus atrophy. Over the past ten years, however, several conditions have
15 become common that require regeneration of the T cell arm of the immune system. These conditions include bone marrow transplantation for a variety of illnesses, cancer chemotherapy for the treatment of various malignancies, and the acquired immune
20 deficiency syndrome (AIDS) resulting from infection with HIV (reviewed in Haynes and Hale, Hospital Practice, 34:59-89 (1999)).

The causes of thymic atrophy during aging in humans are not known, but one postulated cause is
25 aging of the stem cell population of bone marrow cells that migrate to the thymus and give rise to immature then mature T cells. Aging of the thymic

microenvironment (Leiner et al, Immunobiol. 167:345-358 (1984); Utsuyama et al, Mechanisms of Aging and Development 58:267-277 (1991)), which makes a plethora of hematopoietic cytokines (e.g., G-CSF, M-CSF, GM-CSF, LIF, IL-1, IL-6 and TGF-alpha (Haynes et al, 5 Seminars in Immunol. 2:67-77 (1990); Haynes, Thymus 16:143-157 (1990); Le et al, Res. Immunol. 141:271-275 (1990); Le, J. Exp. Med. 174:1147-1157 (1991))), is another postulated cause of thymic atrophy.

10 Studies based on transgenic mice engineered to overexpress specific cytokines in thymus have suggested that, in mice, dysregulated cytokine expression in thymus may participate in, or cause, thymic atrophy. For example, construction of
15 transgenic mice that overexpress stem cell factor (Kapur et al, Blood 90:3018-3026 (1997)), leukemia inhibitory factor (LIF) (Shen et al, EMBO Journal 13:1375-1385 (1994)), oncostatin M (Malik et al, Mol. and Cell. Biol. 15:2349-2358 (1995)), IL-10 (Groux et
20 al, J. Immunol. 162:1723-1729 (1999)), IL-13 (Emson et al, J. Exp. Med. 188:399-404 (1998)) and IFN-gamma (Young et al, Blood 89:583-595 (1997)) leads to thymic atrophy and/or dysfunction. However, thymic
25 dysfunction has also been reported when lack of expression of certain of these same cytokines has been induced by making cytokine "knock-out" mice, as was the case in LIF knock-out mice (Escary et al, Nature 363:361-364 (1993)). LIF knock out mice have a

thymocyte signaling defect in that mature thymocytes do not respond to allogenic antigens. Thus, even though overproduction of certain cytokines in transgenic mice induces thymic atrophy, it is not clear that this is a factor in the aging human thymus.

Similarly, several cytokines have been reported to be trophic for thymopoiesis, including IL-1 and GM-CSF (Kurtzberg et al, PNAS 86:7575-7579 (1989)), IL-7 (Plum et al, Blood 88:4239-4245 (1996); Abdul-Hai et al, Experimental Hematol. 24:1416-1422 (1996)), human growth hormone (Beschorner et al, Transplantation 52:879-884 (1991); Murphey et al, PNAS 89:4481-4485 (1992)), insulin like growth factor-1 (Beschorner et al, Transplantation 52:879-884 (1991)) and IL-11 and IL-3 (Frasca, Intl. Immunol. 8:1651-1657 (1996)). Administration of either IL-7 or IL-11 and IL-3 *in vivo* to mice has been suggested to stimulate reconstitution of T cells after irradiation (in the case of IL-11 and IL-3 or bone marrow transplantation (in the case of IL-7)). Mice with a spontaneous mutation in the M-CSF gene that knocks it out and causes osteopetrosis (op/op mice) also display thymic atrophy and other abnormalities (Philippart and Dourov, N. Path. Res. Pract. 191:499-505 (1995)). Thus, both overexpression and underexpression of certain cytokines have been suggested to be associated with thymic atrophy.

SUMMARY OF THE INVENTION

The present invention relates to a method of reversing thymic atrophy associated aging and to a method of stimulating the thymus for enhanced reconstitution of the T cell arm of the immune system. The invention also relates to a method of treating T cell lymphopenia associated, for example, with autoimmune disease. These methods comprise administering underproduced cytokines, or agonists thereof and/or administering antagonists of overproduced cytokines.

Objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Analysis of the percentage of the thymus in true thymic epithelial space (TES) versus perivascular space. Hematoxylin and eosin stained slides, or slides immunostained with anti-keratin antibodies, were graded for the percent of the thymus tissue containing TES. Data are expressed as % TES versus age.

Figure 2. Number of T cell receptor excised circles (TRECs) present in normal human thymus versus thymus age. Data are number of TRECs per mg thymus tissue. TRECs are signal joint T cell receptor alpha

TRECs measured by the assay of Douek et al (Nature 396:690-695 (1998)). The presence of TRECs in thymus is an indication of ongoing thymopoiesis.

Figures 3A-3D. Steady state changes in normal
5 thymus cytokine mRNA levels during aging. Thymuses
were grouped in the following age quintiles: 0-1 yr,
2-10 yrs., 11-25 yrs., 26-49 yrs., >50 yrs. Thymus
mRNA was extracted and RNase protection assays for
steady state mRNA levels were performed per guidelines
10 of the Pharmingen RiboQuant™ RNase protection assay
for multiplex analysis of cytokine mRNA.

Figure 4. Peripheral CD4, CD45RA+, CD62L+ (naïve
phenotype) cells in the peripheral blood of control
normals, non-thymectomized myasthenia gravis (MG)
15 patients and thymectomized MG patients. Non-
thymectomized MG patients had lower CD4+, CD45RA+,
CD62L+T cells than thymectomized MG patients,
particularly in the 26-49 yr. old age.

Figure 5. Administration of LIF or oncostatin M
20 causes peripheral lymphopenia in mice. The indicated
cytokines were administered according to the protocol
in Table 2, and after 3 days of cytokine injections,
the peripheral blood was taken and total peripheral
lymphocyte counts performed. Both LIF and OSM

significantly suppressed peripheral lymphocyte counts compared to control saline-injected mice ($p < .05$).

Figures 6A and 6B. Cytokine production by anti-CD3 stimulated thymocytes from C57BL/6, CD7⁻, CD28⁻ and CD7/CD28-deficient mice ($n=3$). Thymocytes were cultured at 10^6 /ml with or without 20U/ml rmIL-2 in plates pre-coated with PBS or $10\mu\text{g/ml}$ anti-CD3 for 72 hours. Fig. 6A. IFN- γ . Fig. 6B. TNF- α . $*p \leq 0.05$.

Figures 7A and 7B. LIF induced a decrease in absolute number of murine thymocytes per fetal thymus lobe in FTOC (Fig. 7A) as well as decreased the absolute number of CD4/CD8 DP thymocytes per fetal lobe (Fig. 7B). Day 14 (gest.) fetal thymus lobes were cultured for 7 days and then harvested for cell counts and phenotype by flow cytometry.

Figures 8A and 8B. *In vivo* plasma corticosterone (CS) levels in cytokine treated Balb/c mice ($n=3$). Fig. 8A. {LIF and SCF induced acute rises in CS following a single cytokine dose ($2\mu\text{g}$). Fig. 8B. SCF treatment 3x a day for 3 days induced a significant decrease in plasma CS levels. $*p \leq 0.05$.

Figure 9. LIF-induced thymus atrophy *in vivo* is partially blocked by metyrapone (met) (30mg/kg), an inhibitor of corticosterone production. Female Balb/c

mice (3 per group) were injected i.p. 1x a day for 3 days with saline or metyrapone and 3x a day for 3 days with saline or LIF.

Figures 10A-10D. LIF inhibits thymopoiesis in human thymic organ culture (TOC). Postnatal human thymus tissue (~40mg/treatment) was cultured for 7 days and harvested for cell counts, TREC analysis and phenotyping by flow cytometry. Fig. 10A. LIF treatment (10ng/ml) decreased the number of TRECs/ 1μ g thymocyte DNA in TOC. Fig. 10B. LIF induced a decrease in absolute number of CD4/CD8 DP thymocytes/mg tissue. Fig. 10C. Absolute number of CD4 or CD8 SP thymocytes/mg tissue. Fig. 10D. Absolute number of CD4/CD8 DN thymocytes/mg tissue.

Figure 11. Steady-state mRNA for LIF is elevated in thymus in LPS (100 μ g i.p.) treated Balb/c mice. Total RNA was extracted from mouse thymus at the identified time points after LPS treatment and RNase Protection assay used to determine the level of LIF mRNA as a percentage of GAPDH. Data are mean \pm SEM (n=3). *p \leq 0.05.

Figure 12. Pathways of thymic atrophy. PVS = perivascular space.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates, generally, to a therapeutic strategy that can be used to reconstitute the T cell arm of a mammalian (e.g., human) immune system in those diseases or conditions in which the T cell pool is damaged (e.g., aging, bone marrow transplantation, cancer chemotherapy, AIDS, etc.).

The present invention results, at least in part, from observations made during studies of the cytokine profile in mammalian (i.e., human) thymus tissue from birth to age 78. In these studies, Rnase protection assays were used to quantitate levels of steady state cytokine mRNA levels. Levels of mRNA for various cytokines were correlated, not only with age, but with the levels of thymic epithelial space (the area in which new T cells are capable of being produced) present in each thymus. As a result, a series of cytokines have been identified that are overproduced in the aging thymus (stem cell factor, oncostatin M, LIF, IL-6 and M-CSF). IFN- γ is overproduced in the thymus of MG patients. In addition, various IL-6 family members (e.g., ciliary neurotrophic factor and cardiotrophic factor(CT1)) can also be expected to induce thymic atrophy. In addition, cytokines have also been identified that are expressed in the young thymus when thymopoiesis is greatest, but cease to be expressed in the aged thymus (IL-2, IL-13, IL-9, IL-

10, IL-14). The identification of these over- and underexpressed cytokines makes possible treatment strategies that are designed to increase the levels of certain trophic factors (IL-2, IL-13, IL-9, IL-10, IL-14) and/or diminish the levels of certain other cytokines that inhibit thymopoiesis (stem cell factor, oncostatin M, LIF, IL-6 and M-CSF).

Interestingly, IL-7 mRNA levels did not change over time in the thymic microenvironment, demonstrating that IL-7 is present in the human atrophic thymus, and suggesting an active suppression of thymopoiesis by overproduced cytokines. Indeed, when the overproduced cytokines were administered to mice, each one caused varying degrees of thymic atrophy (Table 2). These data suggest that reversal of thymic atrophy may require a combination of both administration of trophic cytokines and inhibition of overproduced inhibitory cytokines.

Thus, in one embodiment, the present invention relates to a method of reversing thymic atrophy, for example, that associated with aging, stress, AIDS, cancer chemotherapy, irradiation and in the setting of bone marrow transplantation. The method comprises administering to a patient in need thereof an agent that inhibits production and/or function of an overproduced cytokine (that is, an antagonist of the overproduced cytokine) and/or administering an underproduced cytokine or an agent that enhances the

production and/or function of the underproduced cytokine (that is, an agonist of the underproduced cytokine).

Agents suitable for use in inhibiting production
5 and/or function of an overproduced cytokine of the invention include antibodies to the overproduced cytokine, soluble receptors for the overproduced cytokine, and small molecules that mimic the soluble cytokine receptors and that inhibit function of the
10 cytokine. Agents that inhibit expression of overproduced cytokines include anti-sense RNA species that inhibit production/translation of cytokine encoding mRNA. Such agents can be supplied using any of a variety of vectors, including adenoviral and
15 retroviral vectors, naked DNA can also be used.

Antagonists of overproduced cytokines can be administered systemically, orally, or in the subcutaneous tissue, intramuscularly, intranasally or intravenously. Targeted delivery can be effected
20 using thymus-specific ligands. Antagonists can also be administered locally, directly to the thymus, to minimize systemic side effects. Direct administration to the thymus, which can be accomplished using, for example, a mediastinoscope, is preferred when the
25 antagonist is an antisense construct.

Underproduced cytokines of the invention, or agonists thereof, can also be administered systemically, orally, or in the subcutaneous tissue,

intramuscularly, intranasally or intravenously.

Targeted delivery can be effected using thymus-specific ligands. In addition, the underproduced cytokine, or agonist thereof, can be administered

5 directly to the thymus to minimize side effects.

Direct administration to the thymus is preferred when a construct comprising a nucleic acid (e.g., DNA) sequence encoding the cytokine is used to effect expression of the cytokine *in vivo*. Such constructs
10 can be supplied using any of a variety of vectors, including adenoviral and retroviral vectors, naked DNA can also be used.

In a further embodiment, the present invention relates to a method of treating T cell lymphopenia,
15 for example, lymphopenia associated with autoimmune disease. The method comprises administering to a patient in need thereof an agent that inhibits production and/or function of an overproduced cytokine (that is, an antagonist of the overproduced cytokine)
20 and/or administering an underproduced cytokine or an agent that enhances the production and/or function of the underproduced cytokine (that is, an agonist of the underproduced cytokine).

Agents suitable for use in inhibiting production
25 function of an overproduced cytokine of the invention are as described above and include antibodies to the overproduced cytokine, soluble receptors for the overproduced cytokine, and small molecules that mimic

the soluble cytokine receptors and that inhibit function of the cytokine. Agents that inhibit expression of overproduced cytokines are also described above and include anti-sense RNA species
5 that inhibit production/translation of cytokine encoding mRNA.

As above, antagonists of overproduced cytokines can be administered systemically, orally, or in the subcutaneous tissue, intramuscularly, intranasally or
10 intravenously. Alternatively, antagonists can be administered locally, directly to the thymus, to minimize side effects. In accordance with this embodiment also, direct administration to the thymus is preferred when the antagonist is an antisense
15 construct.

In the same manner described above, underproduced cytokines of the invention, or agonists thereof, can be administered systemically, orally, or in the subcutaneous tissue, intramuscularly, intranasally or
20 intravenously. In addition, the underproduced cytokine, or agonist thereof, can be administered directly to the thymus to minimize side effects. Direct administration to the thymus is preferred when a construct comprising a nucleic acid (e.g., DNA)
25 sequence encoding the cytokine is used to effect expression of the cytokine *in vivo*.

It will be appreciated that the agents of the invention can be formulated with appropriate

pharmaceutically acceptable carriers, excipients or diluents to form pharmaceutical compositions. It will also be appreciated that the optimum dosage regimens to be used can be readily determined by one skilled in the art and will vary with the agent, the patient and the effect sought.

It will be further appreciated from a reading of this disclosure that reproducing the normal homeostasis of cytokines within the thymus may require a combination of inhibiting the overproduced cytokines and administering the underproduced cytokines, or agonists thereof, by one of the manners indicated above. For example, IL-7, IL-2 and/or other thymopoietic cytokines can be administered in combination with antagonists of overproduced cytokines.

Certain aspects of the invention are described in greater detail in the non-limiting Examples that follow.

20

EXAMPLE 1

Modulation of T Cell Production Within the Thymus by Cytokines

The decrease in the percent of the whole thymus that the true thymic epithelial space occupies was studied as the thymus aged. The data shown in Figure 1 represent the thymuses tested using RNase protection assays for cytokine mRNA levels (RiboQuant™

RNase protection assay, Pharmingen), that have been analyzed for the percent of the area of the whole thymus that is taken up by keratin and true thymic epithelial space. The data demonstrate that as the
5 human thymus ages, the percent of the whole thymus that contains the true TES (i.e., thymopoiesis) falls to 5% or less by age 78.

To quantitate this decline and to directly measure an indicator of thymopoiesis, these same
10 thymus tissues were analyzed for the presence of T cell receptor excised circles (TRECs). TRECs are made when the T cell receptor alpha chain is rearranged and excises out the T cell receptor (TCR) delta locus, resulting in an episomal circle of DNA that is diluted
15 out in a cell population as the cell divides (Douek et al, Nature 396:690-695 (1998)). Figure 2 shows the TREC levels in whole thymus tissue from birth to 78 years of age expressed as the number of TRECs per mg of tissue. There is a dramatic reduction of
20 thymopoiesis per whole tissue after age forty.

A large panel of cytokines was studied based on the availability of RNase protection assay kits from R and D Systems, Inc. The cytokines are grouped according to whether they were cytokines that, when
25 expressed in a transgenic mouse, caused thymic atrophy (Group A, LIF, oncostatin M, SCF, IL-13), cytokines known to be trophic for T cells (Group B, IL-2, IL-7, IL-15, and IFN- γ), other cytokines known to be made by

thymic epithelial cells (Group C, IL-6, G-CSF, M-CSF) and other immunoregulatory cytokines (Group D, IL-9, IL-10, IL-14).

Figure 3 shows that when the thymuses are grouped in quintiles of age (0-1, 2-10, 11-25, 26-49, >50) the cytokines that significantly increased were stem cell factor, oncostatin M, LIF, IL-6 and M-CSF, and the cytokines that significantly decreased during aging were IL-2, IL-13, IL-9, IL-10, and IL-14. In contrast, IL-7, IL-15, and G-CSF levels did not change during thymus aging. These data are summarized in Table 1 with the associated p values.

Sublethal but large amounts of stem cell factor, oncostatin M, LIF, IL-6 and M-CSF were injected into animals to determine whether such doses of cytokines would cause thymic atrophy. Injection of each of these cytokines, in the dose regimen given in Table 2, induced thymic atrophy compared to control saline injection (Table 2). Thymic weights in LIF, IL-6, M-CSF, oncostatin M and stem cell factor-treated mice were significantly decreased compared to thymus weights of control mice, and total thymocyte numbers were significantly decreased in LIF, IL-6, oncostatin M and stem cell factor treated mice (Table 2).

EXAMPLE 2Modulation of Peripheral T Cell Levels
by the Autoimmune Disease Thymus

Many autoimmune diseases are associated with T
5 cell lymphopenia, such as systemic lupus
erythematosus, myasthenia gravis, and rheumatoid
arthritis (Haynes et al, J. Immunol. 131:773-
777(1983)). The thymus can regulate peripheral T
lymphocyte levels by regulating thymic output of T
10 cells. However, this is rarely observable in adults
due to the extraordinary capacity of the post thymic
peripheral T cell pool to proliferate and reconstitute
the peripheral T cell pool in a thymic-independent
way. A second mechanism of regulating the number of T
15 cells in the circulation is by the effect of humoral
factors on the circulatory and proliferation capacity
of peripheral T cells. One humoral factor well
studied in this regard is serum cortisol levels. High
cortisol levels suppress human peripheral T lymphocyte
20 levels and prevent them from circulating (Fauci, A.,
Annals of Internal Medicine, 84:304-15 (1976)).
Thymectomy of older individuals with myasthenia gravis
(MG) for treatment of their MG resulted in paradoxical
rises in lymphocyte counts (Haynes, J. Immunol.
25 131:773-777 (1983)). This change was attributed to
elevated cortisol levels in those patients with thymic
atrophy and MG; however, it was noted that not all
those who had peripheral T cell rises after thymectomy

had elevated cortisol levels or reductions in their cortisol levels (Haynes, J. Immunol. 131:773-777 (1983)).

T cell levels and the subsets thereof were
5 examined in age matched controls, and in aged matched individuals with MG who had been thymectomized or had not had thymectomy for treatment of MG. Thymectomized MG subjects aged 26-49 yrs. or > 50 yrs were found to
10 have higher peripheral blood T cell levels and higher naive CD4+ T cell levels than their non-thymectomized counterparts (Figure 4), indicating a humoral factor or factors produced by the MG thymus caused or was associated with T cell lymphopenia in MG.

Four patients have been studied before and after
15 thymectomy for MG. In three of the 4 patients, the T cell levels in peripheral blood rose, and in one of 4 patients the peripheral blood T cell level fell after thymectomy (Table 3). The thymuses of these 4 patients at the time of thymectomy were studied
20 histologically, for the presence of thymopoiesis, and cytokine mRNA levels were determined. In the three patients whose peripheral T cell levels rose after thymectomy, little or no thymopoiesis was observed, as measured by the number of double positive cortical
25 thymocytes present. Rather, most of their CD4+ and CD8+ T cells were single positive cells that histologically were located in the non-thymopoietic PVS. In contrast, in the one patient whose T cells

fell after thymectomy, most of the CD4+ and CD8+ T cells were double positive (CD4+ and CD8+) (i.e., immature cortical thymocytes derived from thymopoiesis) and were not single positive (i.e.,
5 mature phenotype, likely derived from peripheral cells or from mature thymocytes that had developed previously but not currently).

The cytokines that were elevated in the thymuses from those patients whose lymphocyte counts rose after
10 thymectomy were similar to those seen in atrophic normal subjects. The main difference in the cytokines of the MG patient who, when their thymus was removed, did not have increases in lymphocyte number, was less elevation of LIF levels.

15 In a further study, large but sublethal doses of stem cell factor, oncostatin M, LIF, IL-6 and M-CSF have been injected into animals and the effect of these doses of these cytokines was observed. Compared to lymphocyte levels in the saline (control) injected
20 mice, mice injected with LIF or oncostatin M according to the regimen shown in Table 2 had significant decreases in peripheral blood lymphocytes (Fig. 5).

EXAMPLE 3

CD7 and CD28 as Regulatory Molecules of Thymic
25 Cytokine Production

Multiple developmentally regulated adhesion molecule-ligand pairs are involved in thymic

epithelial cell (TE)-thymocyte interactions during T cell development. CD165 (AD2) on immature thymocytes and an unknown ligand on TE cells are involved in early T cell maturation (Bruggers et al, J. Immunol. 154:2015 (1995)). CD6 on mature thymocytes and CD166 (ALCAM) on TE cells mediates TE-mature thymocyte binding (Patel et al, J. Exp. Med. 181:1563 (1995)). In addition, CD2 and CD11a/CD18 on thymocytes and CD58 (LFA-3) and CD54 (ICAM-1) on TE cells, respectively, mediate thymocyte binding to TE cells at all stages of thymocyte development (Haynes et al, Seminars Immunol. 2:67 (1990)). These studies have led to the concept that ligation of thymic stromal and thymocyte adhesion molecules in the course of thymic stromal-thymocyte binding induces thymic stromal and thymocyte cytokine production. This has been demonstrated specifically for TE cell IL-1 production (Lee et al, J. Immunol. 144:4541 (1990)).

The role of CD28 as a key T cell costimulatory molecule is well established. The ligands for CD28 are the B7-1 and B7-2 molecules (CD80/CD86) (June et al, Immunol. Today 15:321 (1994)). Ligation of T cell CD28 by CD80 on antigen presenting cells (APC) upregulates CD40 ligand on T cells that bind to CD40 on APC. Thus, ligation of CD28 by B7 molecules enhances T cell cytokine production and maximizes T cell activation. CD28 is an Ig superfamily molecule expressed at low density on most immature thymocytes

and at higher density on mature thymocytes. The CD28 ligand B7-2, is strongly expressed on medullary thymic epithelial cells in postnatal human thymus.

Human CD7 is a 40kDa member of the Ig superfamily
5 that is expressed on most T and NK cells as well as on bone-marrow derived T, B, NK and myeloid precursors (Sempowski et al, Crit. Rev. Immunol. 19:331 (1999)). Immobilized anti-CD7 in the presence of sub-mitogenic anti-CD3 mab induces a co-mitogenic signal leading to
10 T cell proliferation, IL-2 production, and IL-2 receptor expression (Jong et al, Cell. Immunol. 141:189 (1992)). Triggering of CD7 on TCR $\gamma\delta$ + T cells leads to cell activation and induction of TNF- α , TNF- β and GM-CSF mRNA (Carrel et al, Eur. J. Immunol.
15 21:1195 (1991)). Activation of CD7 on NK cells leads to proliferation, IFN- γ production, increased ability to kill NK targets and increased cell adhesion to fibronectin via β 1 integrins (Rabinowich et al, J. Immunol. 153:3504 (1994)).
20 Crosslinking of either CD7 or CD28 on peripheral TCR $\alpha\beta$ + T cells also induces association of the cytoplasmic domains of CD7 and CD28 with phosphatidylinositol (PI)-3-kinase and modulates T cell adhesion. Both CD7 and CD28 molecules are
25 important in host defense, in that CD7 as well as CD28 deficient mice are resistant to LPS-induced shock syndromes and have decreased in vivo IFN- γ and TNF- α production in this setting.

Since T cell development was normal in both CD7 and CD28 deficient mice, CD7/CD28 double deficient mice were made to determine if deficiency in both molecules would alter intrathymic T cell development.

5 However, in CD7/CD28 double-deficient mice, T cell development was normal, and neither CD7 nor CD28 signalling was centrally involved in the early stages of thymocyte development (Lee et al, J. Immunol. 160:5749 (1998)). Rather, it was found that CD7/CD28
10 double-deficient mice had decreased thymocyte proliferative responses to Con A stimulation, and decreased thymocyte and splenocyte IFN- γ and TNF- α production following TCR/CD3 triggering (Figure 6). CD7-deficient mice also had these defects, and the
15 magnitude of the triggering and cytokine production defects were augmented by the combination of CD7 and CD28 deficiencies.

Marine et al. (Cell 98:609 (1999)) have recently observed that mice deficient in suppressor of cytokine
20 signalling-1 (SOCS-1) leads to deregulated IFN- γ production by thymocytes that causes IFN- γ -induced thymic atrophy. Human MG thymuses have accelerated thymic atrophy of aging compared to normal thymuses, and MG thymuses have overexpression of IFN- γ mRNA
25 compared to age-matched normal thymuses (Sempowski et al, J. Immunol. 164:2180 (2000)). Thus, when overexpressed in thymus, IFN- γ is another thymosuppressive cytokine.

EXAMPLE 4

The Role of Corticosteroids in LIF-Induced
Acute Thymic Atrophy

A critical observation has been that LIF, OSM,
5 IL-6 and SCF each induced acute thymic atrophy when
injected *in vivo* in mice (Sempowski et al, J. Immunol.
164:2180 (2000)). A key question is whether
thymosuppressive cytokines directly induce thymocyte
death or indirectly induce thymocyte death via other
10 mediators. Experiments on mechanisms of LIF-induced
acute thymic atrophy address this question. First, it
was determined that LIF, when added in a wide dose
range (.01 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$) to human or mouse PBMC
cultures, neither induced T cell activation, nor
15 inhibited T cell activation by ConA or CD3/TCR
triggering. Further, when added directly to thymocyte
suspensions for 1 to 7 days, LIF neither induced
murine or human thymocyte apoptosis nor necrosis *in*
vitro. However, when LIF was added to 7-10 day murine
20 fetal thymic organ cultures (FTOCs), LIF did induce
thymocyte death (Figure 7). Taken together, these
data demonstrated that the *in vivo* effect of LIF on
thymocyte death was not a direct effect of LIF on
thymocytes, but rather was an indirect effect mediated
25 via a thymus-produced factor.

Melmed et al have demonstrated that hypothalamic
and pituitary LIF, regulated by the SH2-binding
protein SOCS-3 and the protein tyrosine phosphatase,

SHP-1, synergizes with CRH to upregulate pituitary proopiomelanocortin (POMC) gene expression, and thus induce pituitary ACTH production (Shimon et al, J. Clin. Invest. 100:357 (1997); Bousquet et al, J. Clin. Invest. 104:1277 (1999); Bruggers et al, J. Immunol. 154:2012 (1995)). Thus, hypothalamic and pituitary LIF, with hypothalamic CRH, synergize to induce systemic corticosteroid production. Wang has shown that both lipopolysaccharide (LPS) as well as physical stress (restraint and immobilization) induce pituitary and hypothalamic LIF production (Wang et al, Endocrinol. 137:2947 (1996)). One possible mechanism of LIF-induced acute thymic atrophy is via stimulation of pituitary ACTH to raise systemic corticosteroid levels. Plasma corticosterone levels have been measured 2 hours after IP LIF (2 μ g/mouse) and it was found that, indeed, doses of LIF that induce thymic atrophy, induce elevations in plasma cortiosterone levels (Figure 8A). A second possible mechanism of LIF-induced thymic atrophy is that induction of a factor from thymus mediates thymocyte death. The production of corticosteroids by thymic stromal cells has been demonstrated. Physiologic corticosteroid levels (10^{-9} M) are required for maturation of immature thymocytes and for normal positive and negative thymocyte selection. In contrast, stress and pharmacologic levels (10^{-8} M, 10^{-7} M) of corticosteroids in the thymus induce thymocyte endonucleases and

widespread thymocyte apoptosis and acute thymic atrophy (Wyllie et al, Nature 284:555 (1980)). It has been shown that LIF is produced by thymic epithelial space TE cells (Lee et al, J. Immunol. 145:3310 (1990)) and by adipocytes in the thymic perivascular space (Sempowski et al, J. Immunol. 164:2180 (2000)).

A further questions is whether LIF-induced thymocyte death *in vivo* is inhibited by the corticosteroid-synthesis inhibitor, metyrapone.

10 Metyrapone significantly inhibited LIF-induced thymic atrophy *in vivo* (Figure 9), demonstrating that corticosteroids indeed play a role in mediating LIF-induced thymic atrophy.

It was previously shown that human TE cells were
15 neuroendocrine in origin by expression of surface markers such as complex gangliosides shared with tissues derived from neural crest ectoderm (Haynes et al, J. Clin. Invest. 71:9 (1983)). It has also been demonstrated that production of thymic corticosteroids
20 was by a radioresistant thymus cell (Vacchio et al, J. Exp. Med. 179:1835 (1994)), and Lechner et al. (Eur. J. Immunol. 30:337 (2000)) and Pazirandeh et al (FASEB J. 13:893 (1999)) showed directly that TE cells are the source of thymic corticosteroids in mice.

25 The culture conditions used for murine FTOCs for human postnatal thymus organ cultures (TOCs) were adopted. Figure 10 shows that as in murine FTOCs, human postnatal TOCs treated with LIF also showed

decreased DP thymocytes and decreased TREC+ thymocytes after 7 days in culture. In addition, it was shown that the maintenance or loss of TREC+ DP and SP thymocytes correlated with loss of CD1a+ DP thymocytes, and, taken together, were excellent indicators of loss of thymopoiesis. Thus, the data indicate that *in vivo* LIF induces DP thymocyte apoptosis at least in part via induction of corticosteroids that are inhibited by metyrapone. *In vitro*, LIF had no effect on thymocyte apoptosis in isolated thymocytes in suspension, but did induce thymocyte apoptosis when added to mouse FTOCs and to human postnatal TOCs. In addition, systemic administration of LIF also raised murine plasma corticosterone levels. Thus, LIF may elevate systemic corticosteroid levels, elevate thymic corticosteroid levels or both. It is important to note that for LIF and related cytokines that act through modulation of systemic and/or thymic corticosteroid levels, corticosteroid synthesis inhibitors or corticosteroid receptor inhibitors (either in the thymus or systemically) can be of use in augmenting immune reconstitution of T cells.

EXAMPLE 5

LPS Upregulates Thymosuppressive Cytokine mRNA
Expression in Thymus

It is important to determine the biological
5 relevance of thymosuppressive cytokine-induced acute
thymic atrophy in clinically relevant models of thymic
atrophy. Key to showing the relevance of LIF, OSM,
IL-6 and SCF-induced thymic atrophy, would be to
demonstrate the upregulation of thymosuppressive
10 cytokine mRNA and protein levels in the thymus of
animals injected with LPS. LPS-induced shock is a
relevant model of gram negative bacterial sepsis that
occurs in humans and results in ~80% loss in thymus
cellularity due to LPS induction of thymocyte
15 apoptosis within 48 hrs. of LPS administration.
Studies have been performed on the LPS-induced thymic
atrophy model (Zhang et al, Infect. Immun. 61:5044
(1993); Wang et al, J. Immunol. 152:5014 (1994)) using
RNase protection assays for thymus steady-state mRNA
20 levels for LIF and IL-11. It was found that LPS
induced elevated levels of LIF mRNA 6 hours after
injection of 100 μ g of LPS (Figure 11). No changes in
IL-11 mRNA steady state levels were seen. These were
extraordinary results, and confirmed the induction of
25 elevated LIF thymic mRNA levels in endotoxin shock
mediated by LPS.

It has been shown that LPS induces LIF and LIF
receptor (R) upregulation in hypothalamus and

pituitary, and induces CRH from the hypothalamus leading to synergistic induction of ACTH and rise in systemic corticosteroid plasma levels (Wang et al, Endocrinol. 137:2947 (1966)). Further Zhang et al. (Infect. Immun. 161:5044 (1993)) and Wang et al (J. Immunol. 152:5014 (1994)) have demonstrated that injection of high dose (50-100 μ g) LPS into mice indeed induces thymocyte apoptosis and acute thymic atrophy. A plausible model pathway of induction of acute thymic atrophy that involves LIF as well as other inflammatory cytokines can be constructed. LPS induces both systemic and hypothalamic TNF α , IL-6 and IL-1 α and β , as well as LIF, and Wang et al (J. Immunol. 152:5014 (1994)) and Zhang (Infect. Immun. 161:5044 (1993)) both found that pretreatment of mice with anti-TNF α antibody partially prevented LPS induction of thymic atrophy. However, it was also shown that whereas LIF-deficient animals had markedly diminished ACTH responses to restraint stress, plasma corticosterone levels were elevated in restrained LIF-deficient animals (Chesnokova et al, Endocrinol. 139:2209 (1998)). These latter data demonstrated that LIF alone was not responsible for rises in systemic corticosteroid levels induced by restraint stress. Indeed IL-6, both by acting on the pituitary to induce ACTH, and by direct effects on the adrenal to stimulate corticosteroids, is a mediator of stress steroid responses (Zhou et al, Endocrinol. 133:2523

(1993)). In addition, stress activation of the sympathetic nervous system can directly raise adrenal production of corticosteroids via release of catecholamines (Ehrhart-Bornstein et al, Eur. J. Endocrinol. 135:19 (1996)). Nonetheless, the key role LIF does play in the LPS-induced stress/shock responses was shown by Bloch et al. (J. Exp. Med. 178:1085 (1993)). They demonstrated that pretreatment of mice with neutralizing anti-LIF polyclonal antibody prevented death in the high dose (100 μ g) LPS shock model that is mediated by IL-1 β and TNF- α . However, the thymus was not evaluated in this study. Pretreatment of animals with anti-LIF antibody prior to administration of lethal amounts of LPS, not only protected them from death, but also blunted LPS-induced rises in plasma TNF α and IL-1 β . Taken together, these data indicate suggest that LIF and other as yet unstudied IL-6 family cytokines are likely involved in mediating thymic atrophy in stress responses.

EXAMPLE 6

Effect of Stem Cell Factor (SCF) Administration on Thymic Atrophy and Peripheral Corticosteroid Plasma Levels

SCF is not a member of the IL-6 family of cytokines, and its ability to induce thymic atrophy does not fit into any known paradigm. To begin to

analyze how SCF may modulate thymic cellularity and corticosteroid levels, SCF (2 μ g IP) was injected into mice in doses that produce thymic atrophy the effect on plasma corticosteroid levels determined. Whereas
5 acutely (1 hr. after LPS) SCF and LIF both raised plasma corticosteroid levels (Figure 8A), after 3 days (when plasma corticosteroid levels had returned to normal in LIF-treated mice), plasma corticosteroid levels in SCF-treated mice were unexpectedly
10 suppressed (Figure 8B). Thus, SCF both elevates early (0-2hr) and markedly suppresses late (3 days) plasma corticosteroids by as yet unknown mechanisms. If these changes are also occurring intrathymically, then both SCF-induced early elevation of intrathymic
15 corticosteroids leading to thymocyte apoptosis, and later suppression of intrathymic corticosteroids, leading to lack of thymocyte maturation, could both suppress thymopoiesis.

EXAMPLE 7

20 The Role of Corticosteroids in the Thymic Atrophy of Aging

It is postulated that thymic atrophy of aging is mediated by intrathymic events rather than by systemic stress responses (Figure 12). The hypotheses
25 regarding thymic atrophy of aging are that: 1) intrathymic and/or systemic levels of thymosuppressive cytokines LIF, OSM, IL-6 and SCF rise during aging, 2)

thymosuppressive cytokines stimulate and raise intrathymic and/or systemic corticosteroid levels, 3) elevations of intrathymic corticosteroid levels lead to gradual loss of normal positive selection of thymocytes, 4) increase in intrathymic corticosteroid levels with aging eventually reaches "stress" levels and results in direct corticosteroid induction of thymocyte endonucleases and thymocyte apoptosis and, 4) with aging, elevated LIF levels intrathymically also drive adipocyte differentiation of preadipocytes in the thymic perivascular space to gradually fill the PVS with adipocytes (Figure 12).

It has been shown that thymic stromal cells make corticosteroids that in physiologic amounts modulate the efficiency of positive and negative thymocyte selection (King et al, Ummunity 3:647 (1995); Vacchio et al, J. Immunol. 163:1327 (1999); Vacchio et al, J. Exp. Med. 185:2033 (1997); Vacchio et al, J. Exp. Med. 179:1835 (1994). LIF or other thymosuppressive cytokine-induced elevation in intrathymic corticosteroid production could lead to both decreasing levels of appropriately positively selected thymocytes, and as corticosteroid levels approached supraphysiologic levels in the thymus, could lead to induction of thymocyte death and decrease in thymopoiesis.

Finally, lymphocyte and adipocyte cytokine production in the thymic perivascular space may play

an important role in regulating the rate of thymopoiesis. In this regard, it has been shown that human adipocytes expresses LIF, OSM, IL-6 and SCF mRNA. It has also been shown that preadipocytes make LIF and LIFR, and blocking LIFR prevents preadipocyte differentiation to adipocytes. Preadipocytes differentiated from LIF-deficient ES cells continued to differentiate to adipocytes, indicating that in addition to LIF, other adipocyte-produced IL-6 family cytokines that use the LIFR, such as CNTF or CT-1, also are involved with LIF in driving adipocyte differentiation. LIF-driven adipocyte differentiation is via induction of adipocyte differentiation transcription factors C/EBP β , and C/EBP δ , and activation of MAP kinase via STAT-3. Given that it has been shown that human TE cells and human thymic adipocytes produce LIF and IL-6, thymosuppressive cytokine production may be by TE cells (paracrine), or from adipocytes, or from both sources.

It has been shown that plasma cortisol levels do not rise with aging and progressive thymic atrophy in man, and as well, it has not been possible to detect measurable rises in plasma LIF levels during aging. Therefore, while there may be effects of aging on systemic cytokine and corticosteroid levels, they have not yet been detected. Rather, changes in aged thymus cytokine mRNA levels have been detected. It is hypothesized that with aging as yet unknown

factors/events drive intrathymic thymosuppressive cytokine levels that mediate their effects, at least in part, through local intrathymic increases in corticosteroid production. Moreover, it is likely
5 that some of these same thymosuppressive cytokines (LIF and other IL-6 family members) drive adipocyte differentiation in the thymic perivascular space.

EXAMPLE 8

Study of Functions of CD7 and The Normal Ligand
10 for CD7, K-12

K-12 is an IFN- γ -inducible, intracellular, secreted molecule expressed in spleen, thymus, prostate, breast, testes, small intestine and granulocytes (Slentz-Kesler et al, Genomics 47:327
15 (1998)). The ligand for K-12 was found it to be its nearest neighbor on chromosome 17, CD7 (Lyman et al, J. Biol. Chem. 275:3531 (2000)). It has been shown by RT-PCR that K-12 is expressed by human TE cells and TF cells and is expressed by our transformed TE cell
20 line, TE750.

It has been shown that IFN- γ mRNA is markedly over-expressed in MG thymuses compared to age-matched normal thymuses, and patients with MG have accelerated thymic atrophy compared to normals (Sempowski et al,
25 J. Immunol. 164:2180 (2000)). MG thymuses differ from normal thymus in the degree of infiltration of the perivascular space with peripheral T and B cells, with

MG patients often having PVS infiltration with large B
cell germinal centers. SOCS-1 deficient mice
overexpress IFN- γ in thymocytes that induces thymic
atrophy. It is hypothesized that when overproduced in
5 human thymus, IFN- γ can contribute to induction of
thymic atrophy.

* * *

All documents cited above are hereby incorporated
in their entirety by reference. Also incorporated by
10 reference are the amino acid sequences and encoding
nucleic acid sequences for the cytokines referenced
herein (particularly the human sequences), those
sequences being available through GENBANK.

One skilled in the art will appreciate from a
15 reading of this disclosure that various changes in
form and detail can be made without departing from the
true scope of the invention.

Table 1

Changes in Thymus Cytokine mRNA During Aging

Cytokine	Change During Aging
Leukemia Inhibitory Factor	↑
Oncostatin M	↑
Stem Cell Factor	↑
IL-13	↓
IL-2	↓
IL-7	↔
IL-15	↔
IFN- γ	↔
IL-6	↑
G-CSF	↔
M-CSF	↑
IL-9	↓
IL-10	↓
IL-14	↓

↑ = increases during aging

↓ = decreases during aging

↔ = no changes during aging

Table 2

Administration of Recombinant Leukemia Inhibitory Factor, Oncostatin M, M-CSF,
Stem Cell Factor or IL-6 to Mice Induces Thymic Atrophy

Treatment	Number of Mice Studied	Total Thymus Weight (mg)	Total Thymocytes per organ
LIF 2 μ g IP 3x daily x 3 days	3	18 \pm 3*	10 \pm 7 x 10 ^{6*}
IL-6 3 μ g IP 3x daily x 3 days	3	35 \pm 4*	23 \pm 8 x 10 ^{6*}
M-CSF 1 μ g IP 3x daily x 3 days	3	47 \pm 3*	38 \pm 7 x 10 ⁶
Oncostatin M 2 μ g IP 3x daily x 3 days	3	17 \pm 4*	7 \pm 2 x 10 ^{6*}
Stem cell factor 2 μ g IP 3x daily x 3 days	3	40 \pm 3*	27 \pm 3 x 10 ^{6*}
Saline control injections IP 3x daily x 3 days	3	55 \pm 3	51 \pm 12 x 10 ⁶

* p < 0.05 compared to control mice.

Table 3

Changes in CD4+T Cell Levels in Myasthenia Gravis Patients Undergoing Thymectomy

	Cells/mm ³	
Patient 13	Prethymectomy	93 days Post
Total CD4+T cells	436	988
Patient 29	Prethymectomy	53 days Post
Total CD4+T cells	1097	194
Patient 32	Prethymectomy	67 days Post
Total CD4+T cells	151	3227
Patient 36	Prethymectomy	22 days Post
Total CD4+T cells	1067	1595

WHAT IS CLAIMED IS:

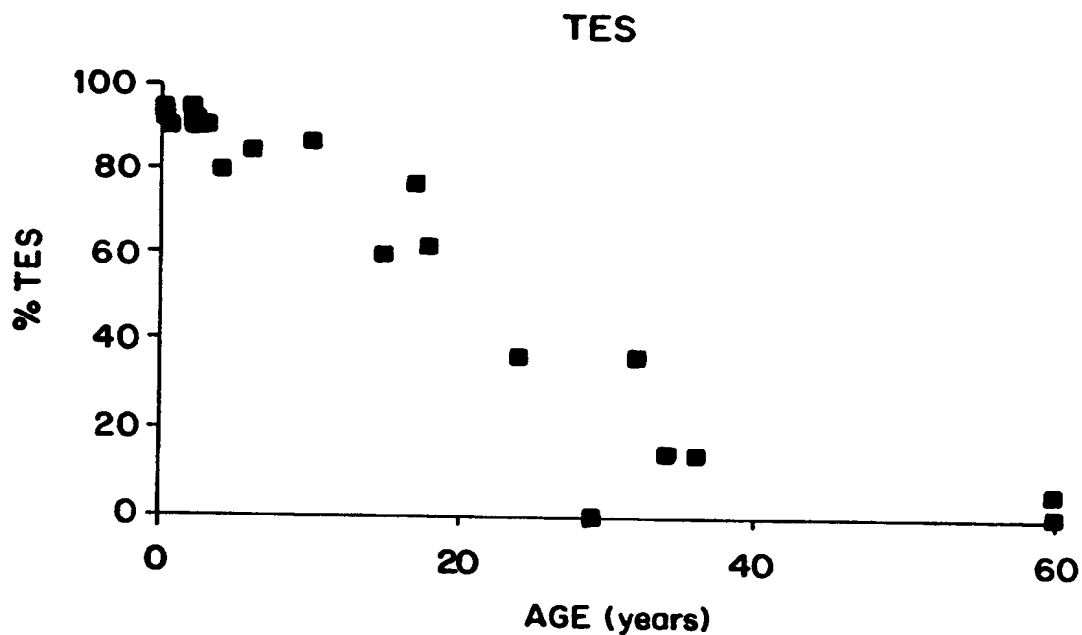
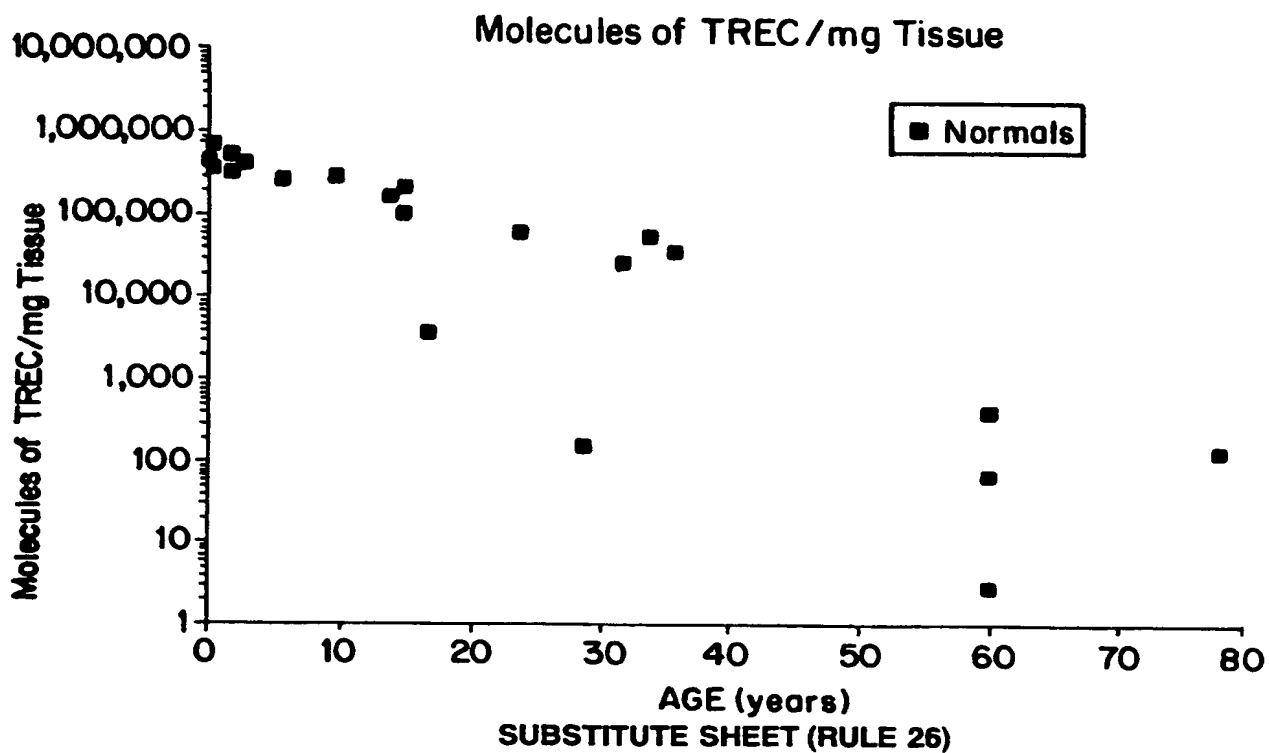
1. A method of reversing thymic atrophy comprising administering to a patient in need thereof an antagonist of an overproduced cytokine in an amount such that said reversal is effected.
2. A method of reversing thymic atrophy comprising administering to a patient in need thereof an underproduced cytokine or agonist thereof in an amount such that said reversal is effected.
3. The method according to claim 1 wherein said antagonist is an antibody to said overproduced cytokine or soluble receptor for said overproduced cytokine or mimic thereof.
4. The method according to claim 1 wherein said antagonist is an anti-sense RNA species that inhibits production or translation of cytokine encoding mRNA
5. A method of treating T cell lymphopenia comprising administering to a patient in need thereof:
i) an agent that inhibits production or function of an overproduced cytokine or ii) an underproduced cytokine or an agent that enhances the production or function

of said underproduced cytokine, in an amount sufficient to effect said treatment.

6. The method according to claim 5 wherein said agent that inhibits production or function of said overproduced cytokine is an antibody to said overproduced cytokine or a soluble receptor for said overproduced cytokine or mimic thereof.

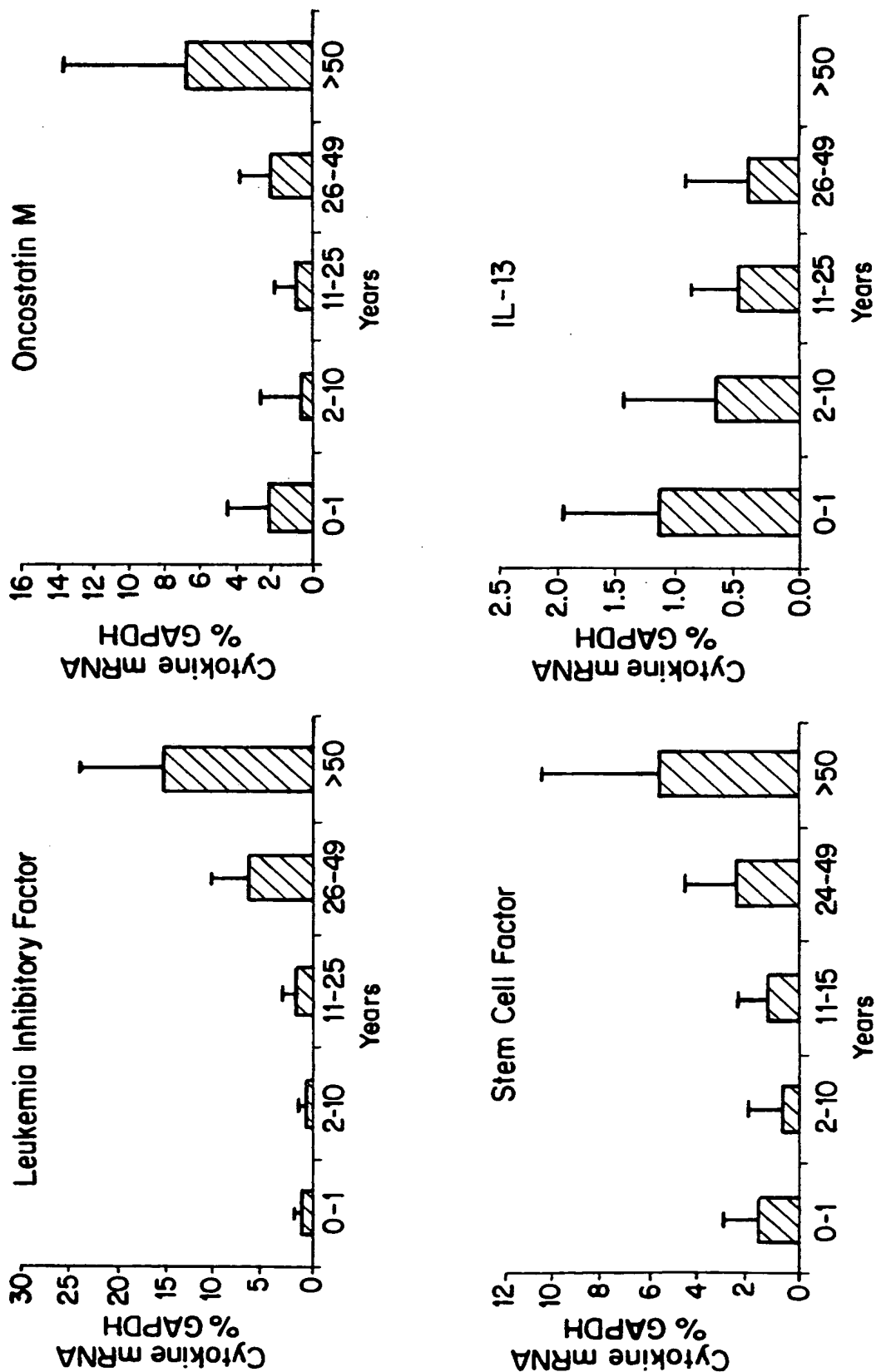
7. The method according to claim 5 wherein said agent that inhibits production of said overproduced cytokine is an anti-sense RNA species that inhibits production or translation of cytokine encoding mRNA.

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FIG. 1**FIG. 2**

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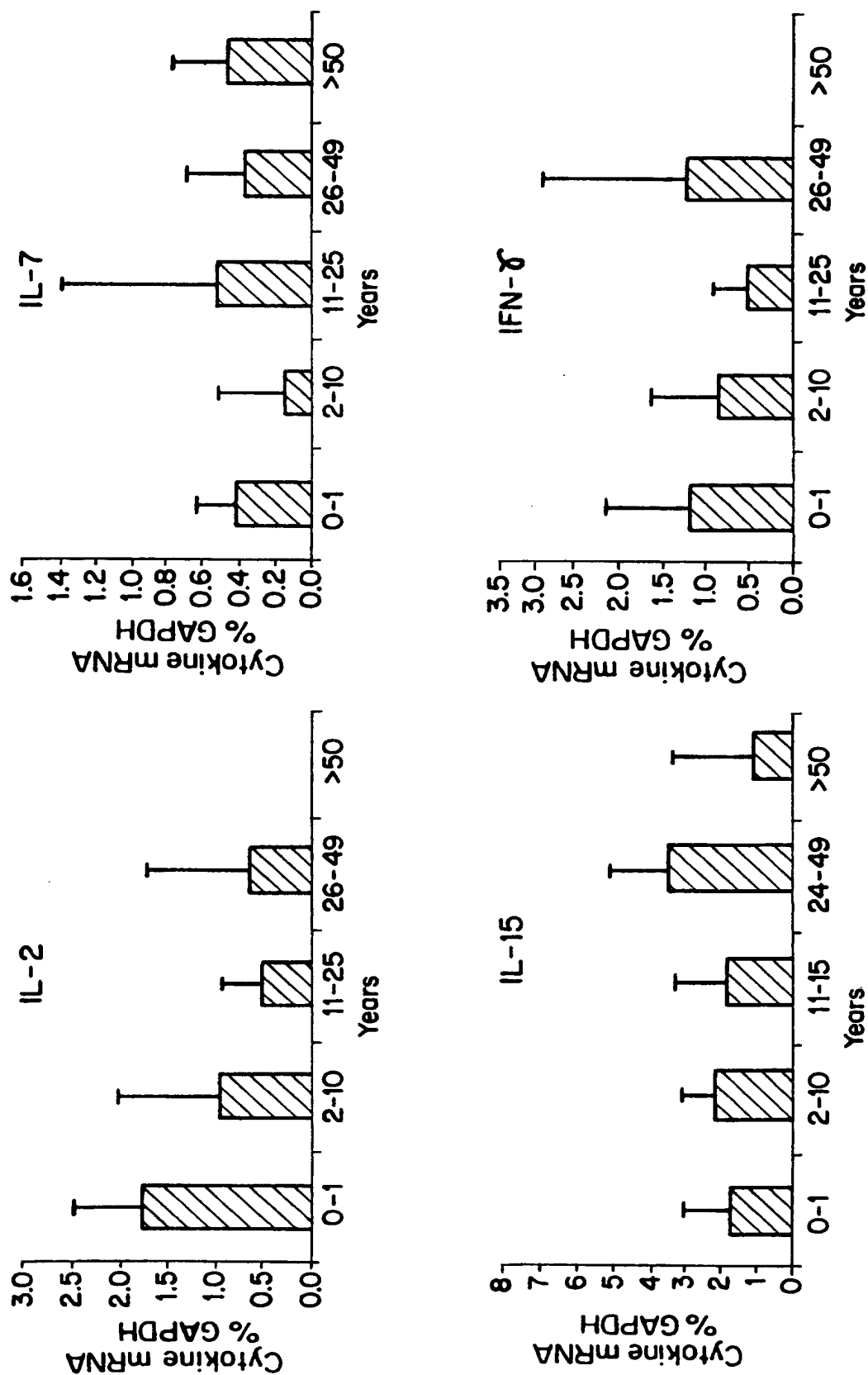
FIG. 3A



(9) SUBSTITUTE SHEET (RULE 26)

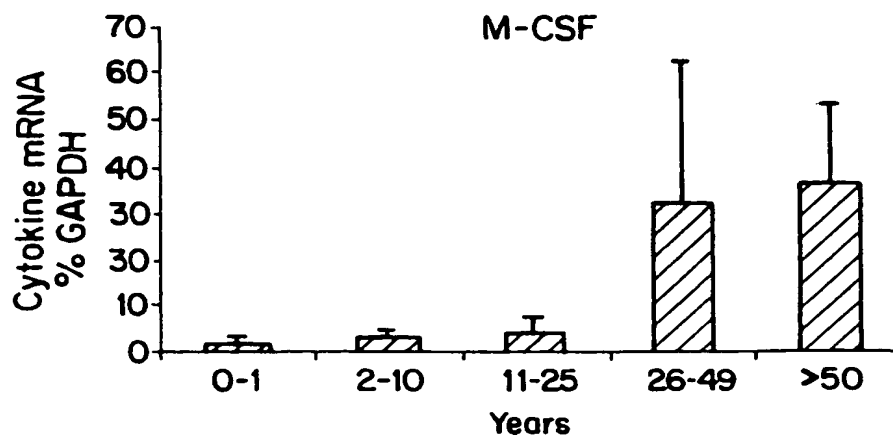
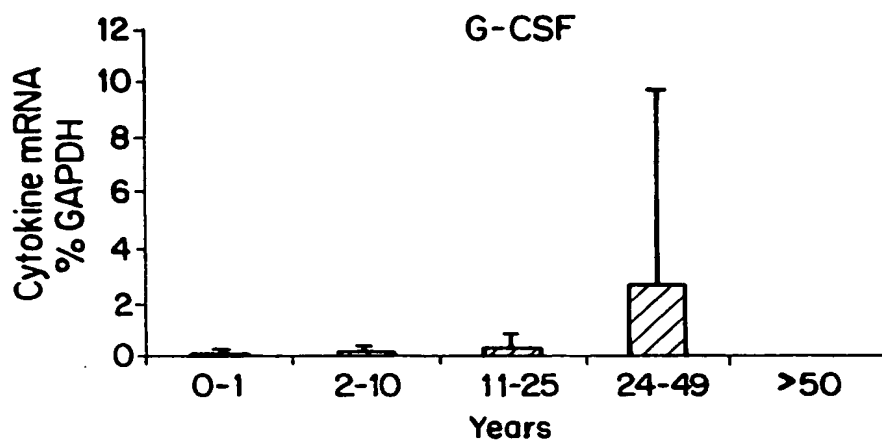
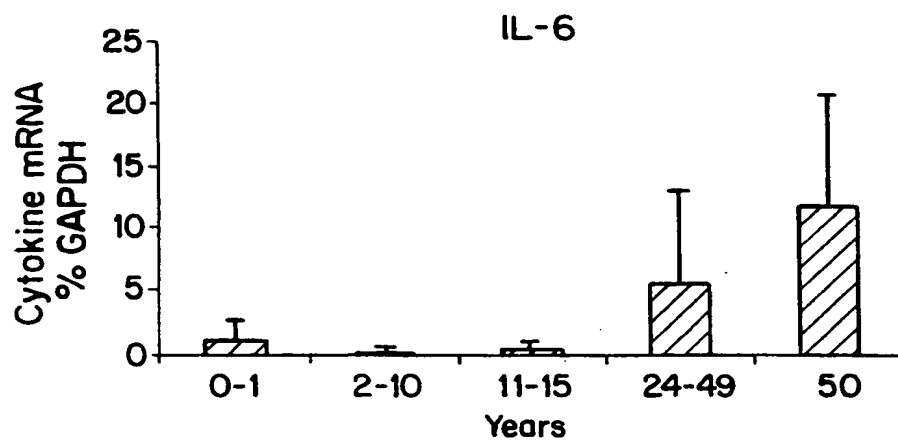
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FIG. 3B



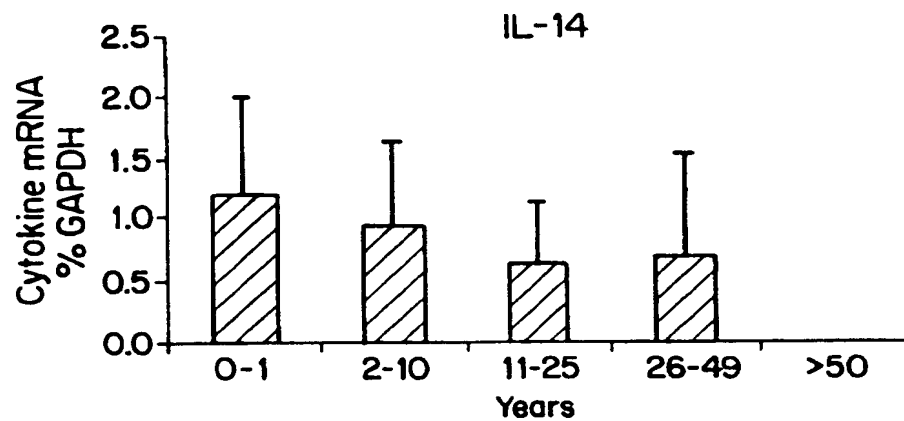
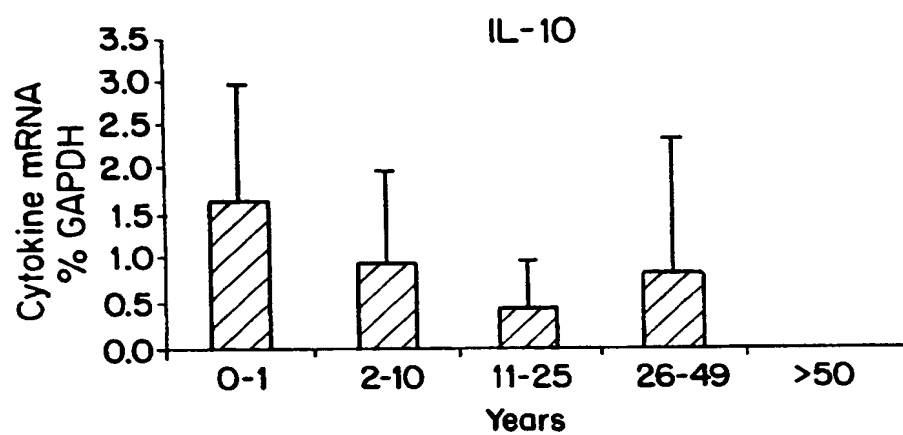
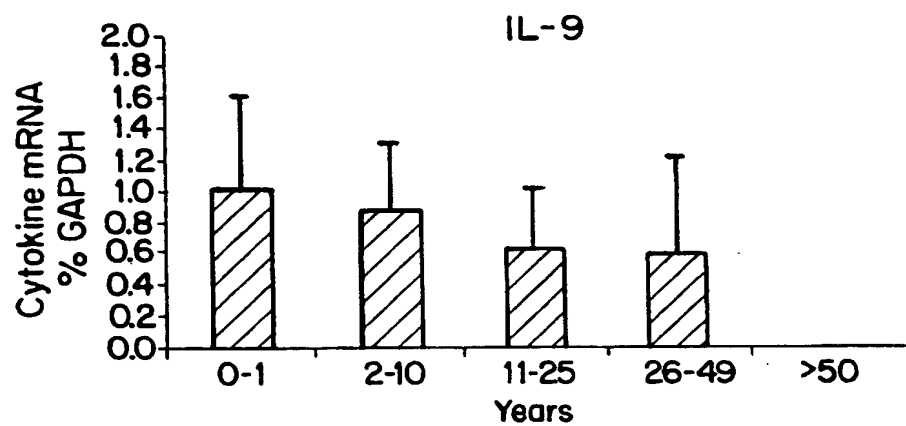
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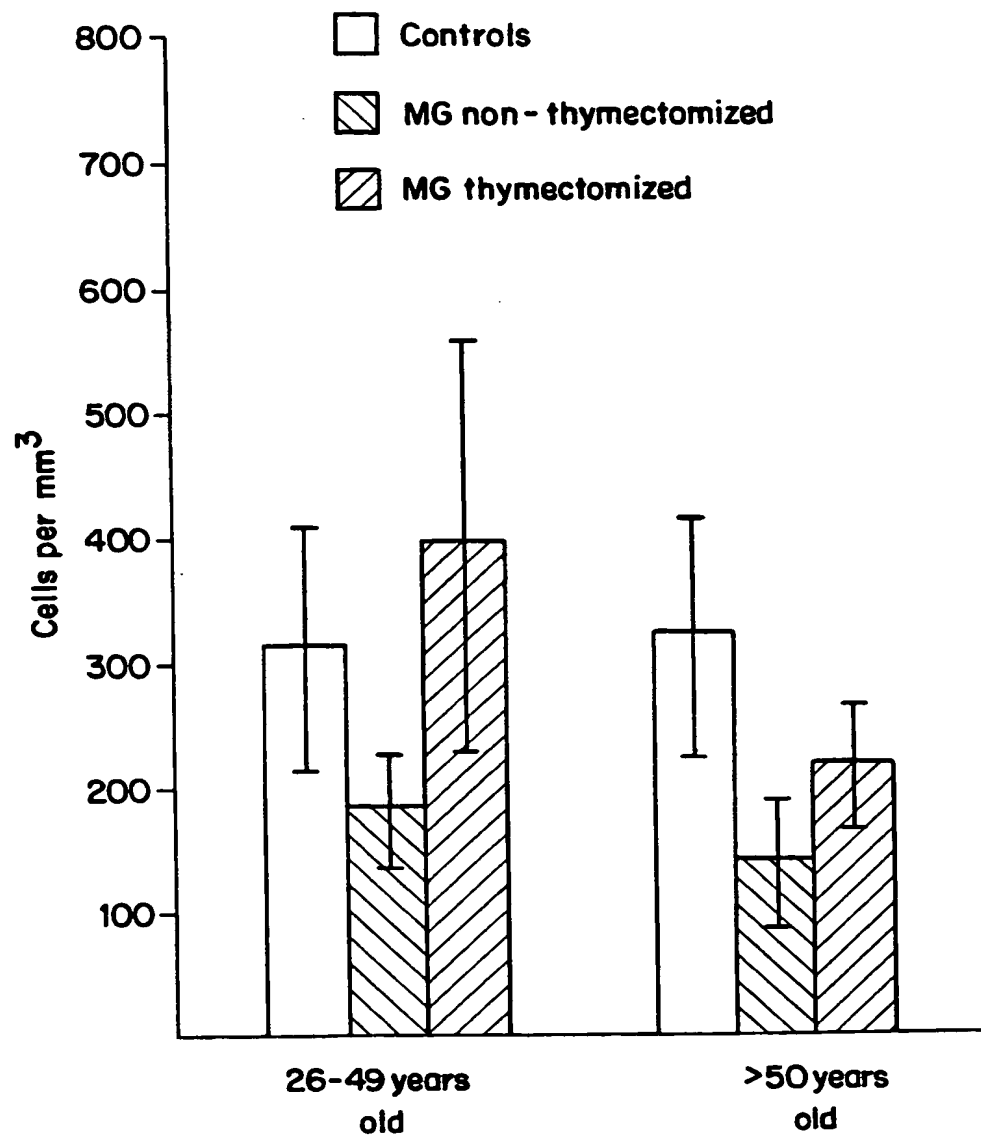
FIG. 3C

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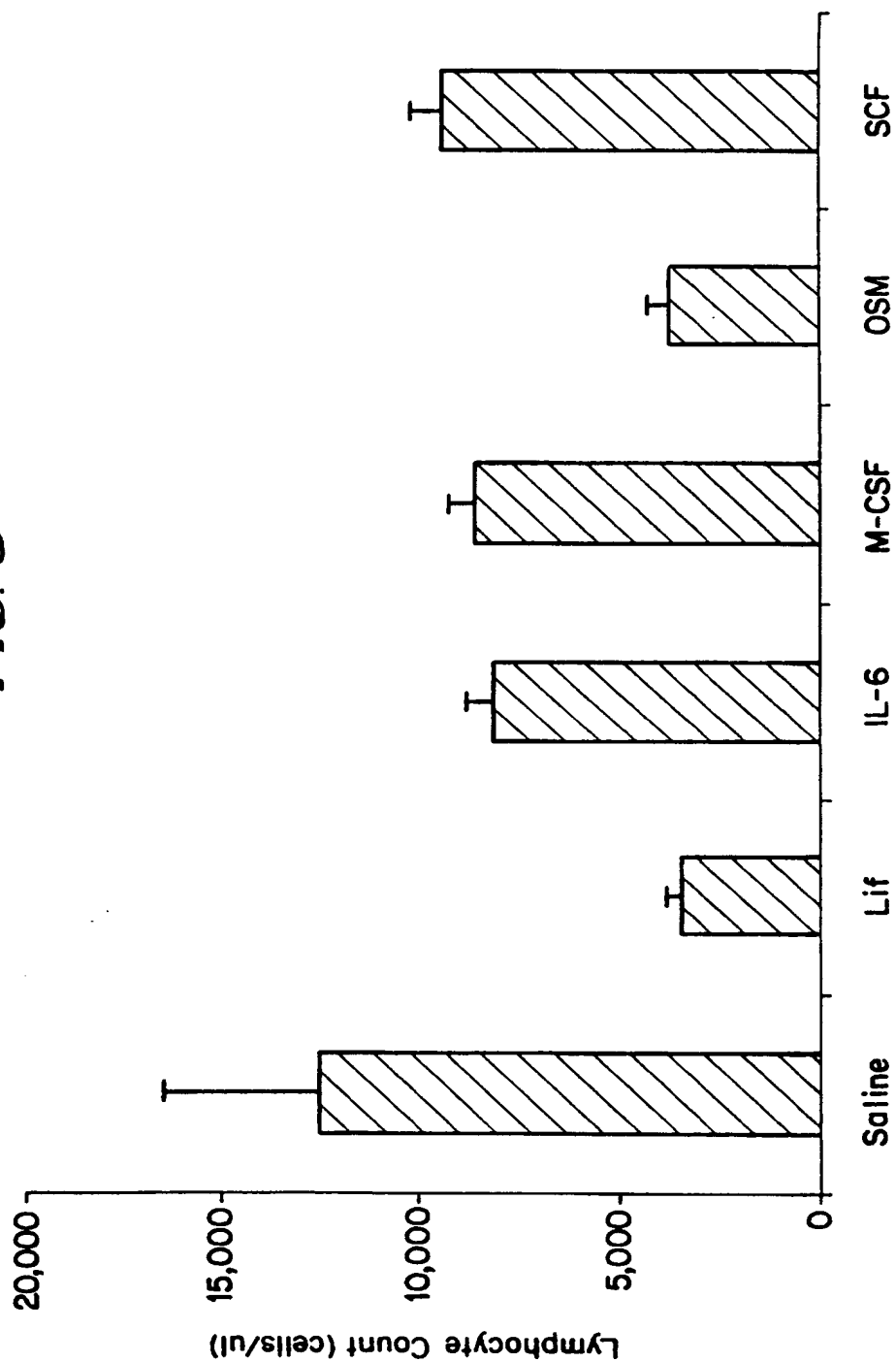
FIG. 3D***SUBSTITUTE SHEET (RULE 26)***

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FIG. 4**CD4⁺, CD45RA⁺, CD62L⁺ T CELLS*****SUBSTITUTE SHEET (RULE 26)***

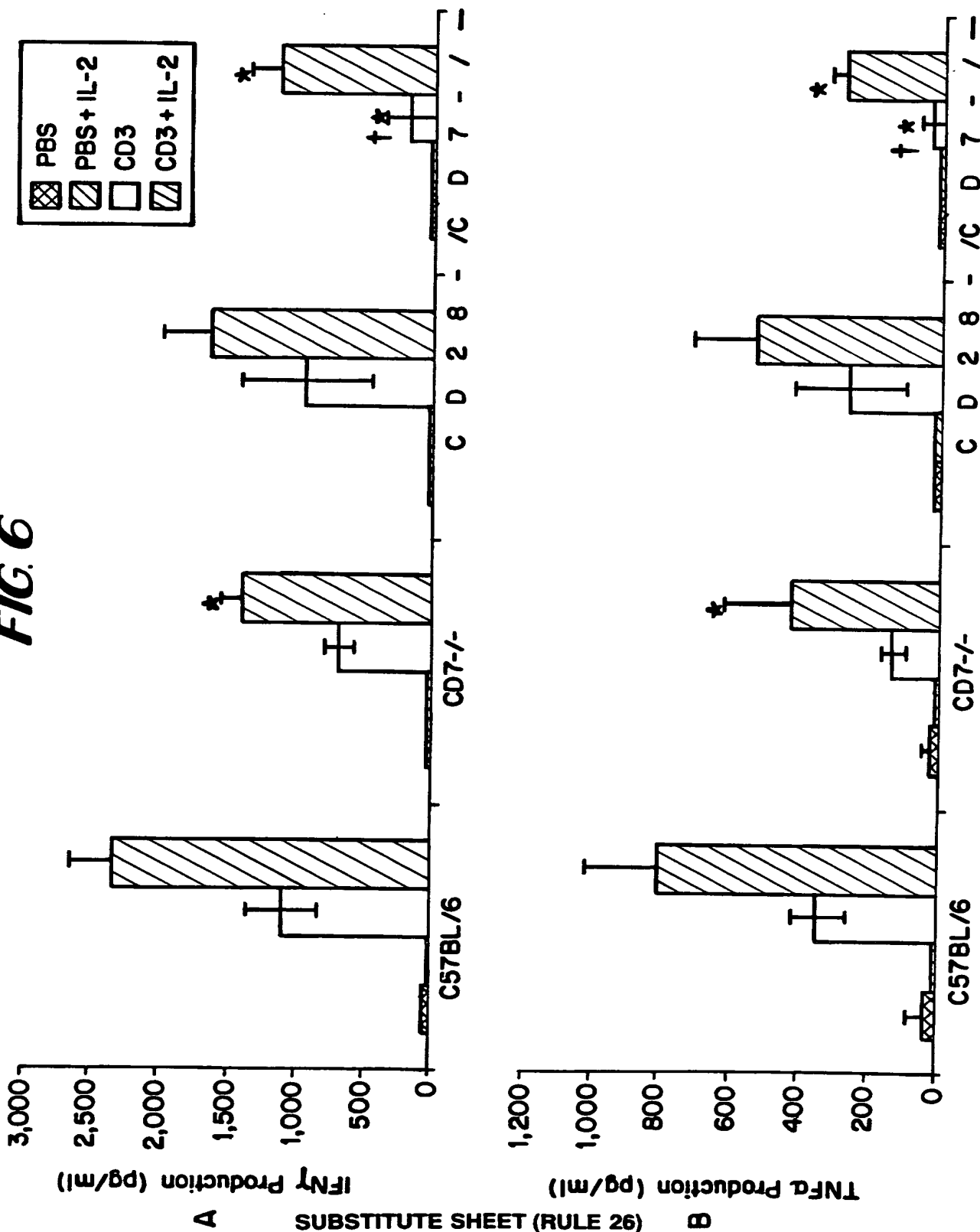
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FIG. 5



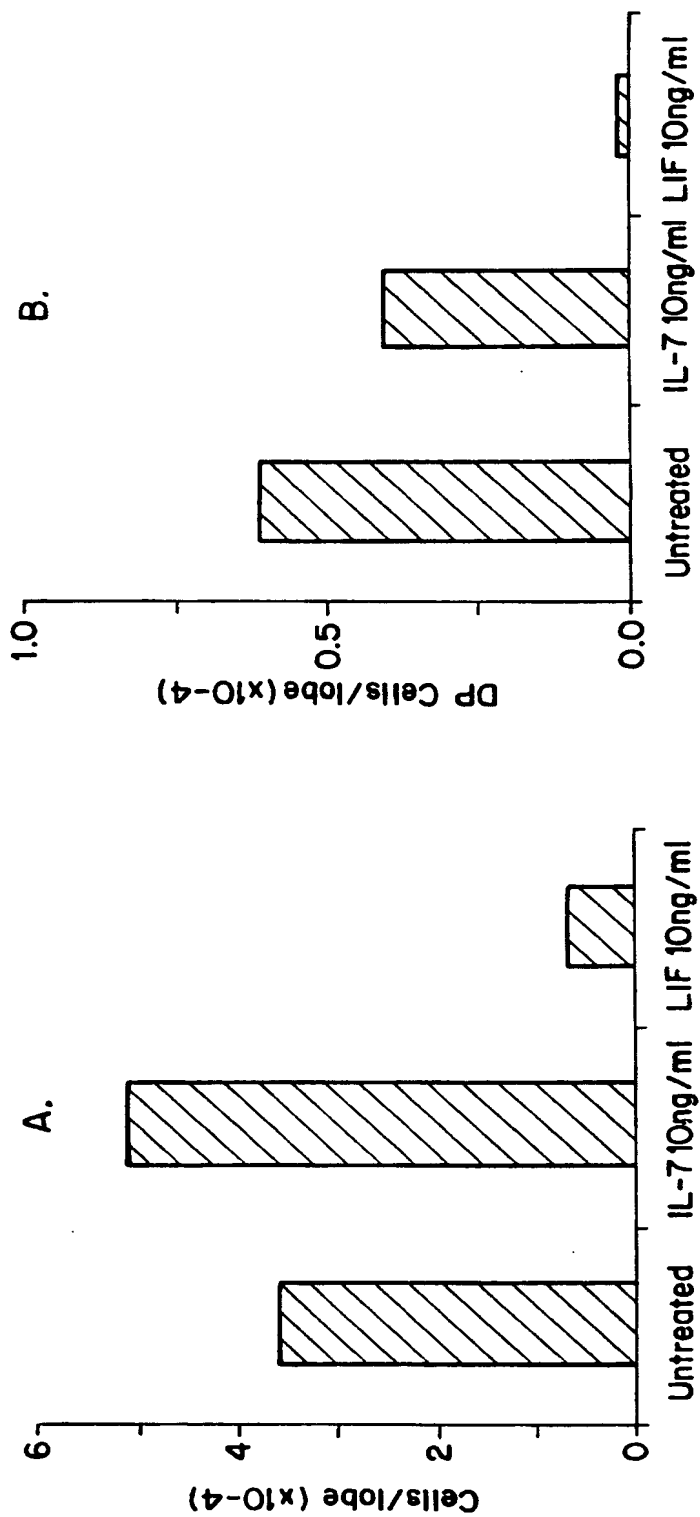
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FIG. 6

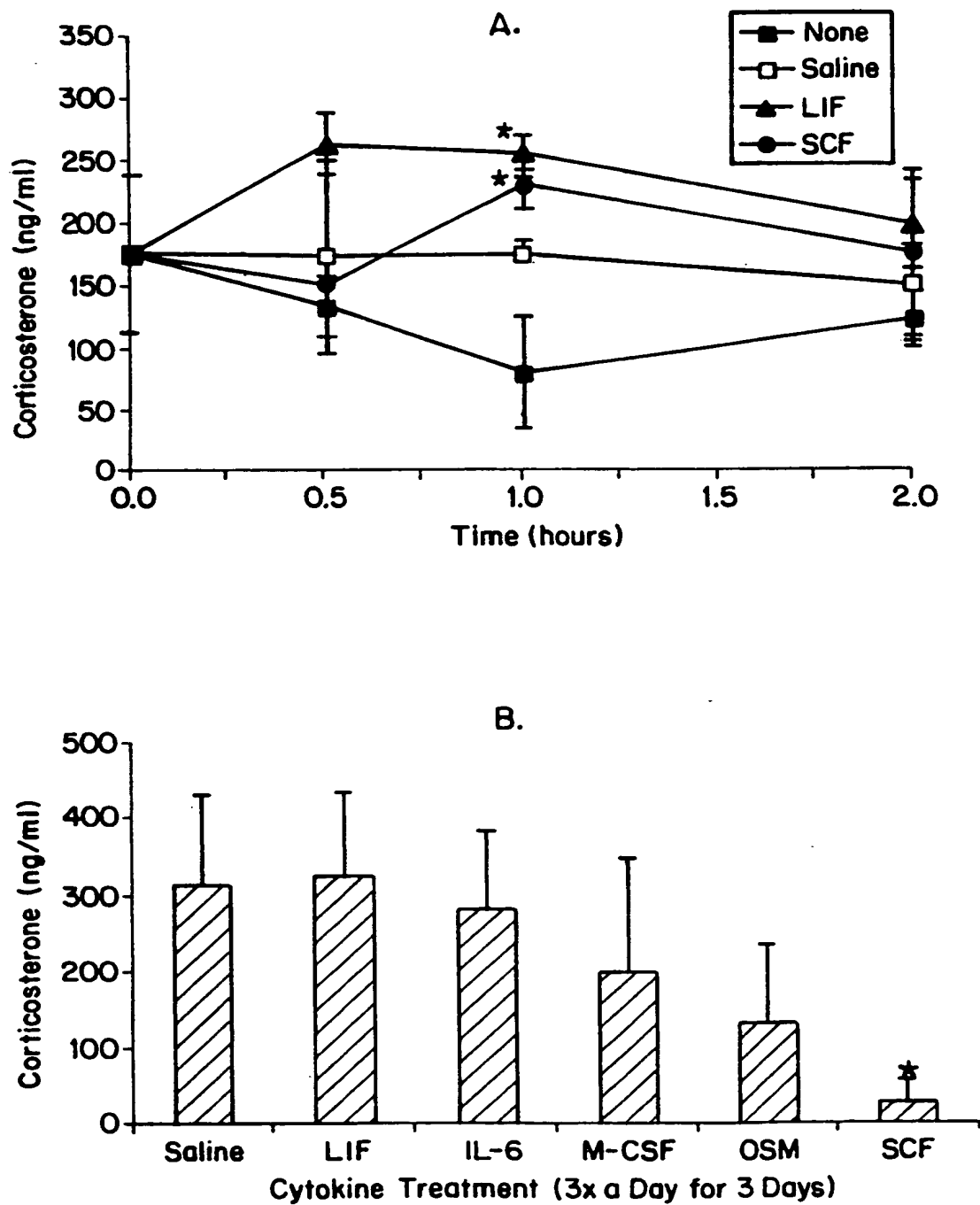


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FIG. 7

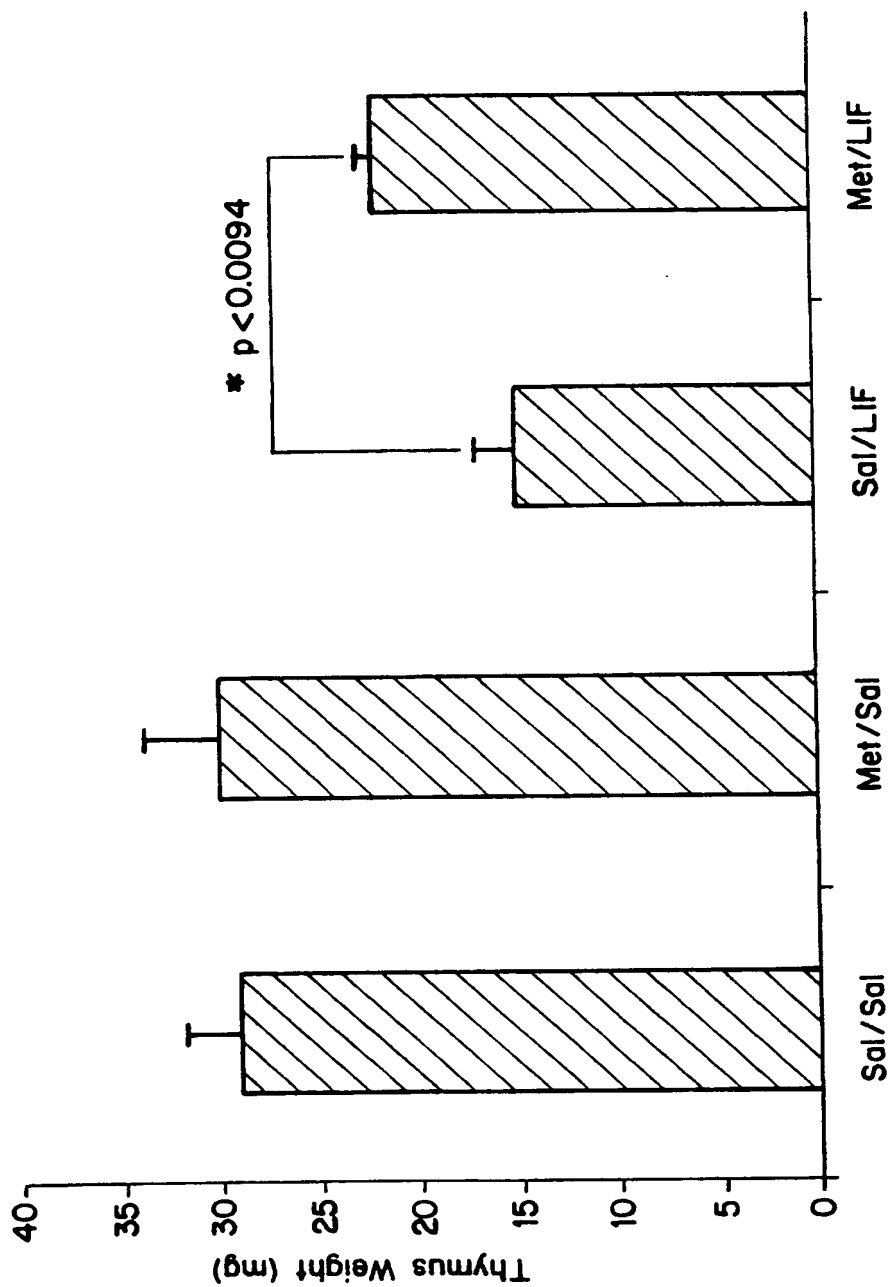


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FIG. 8

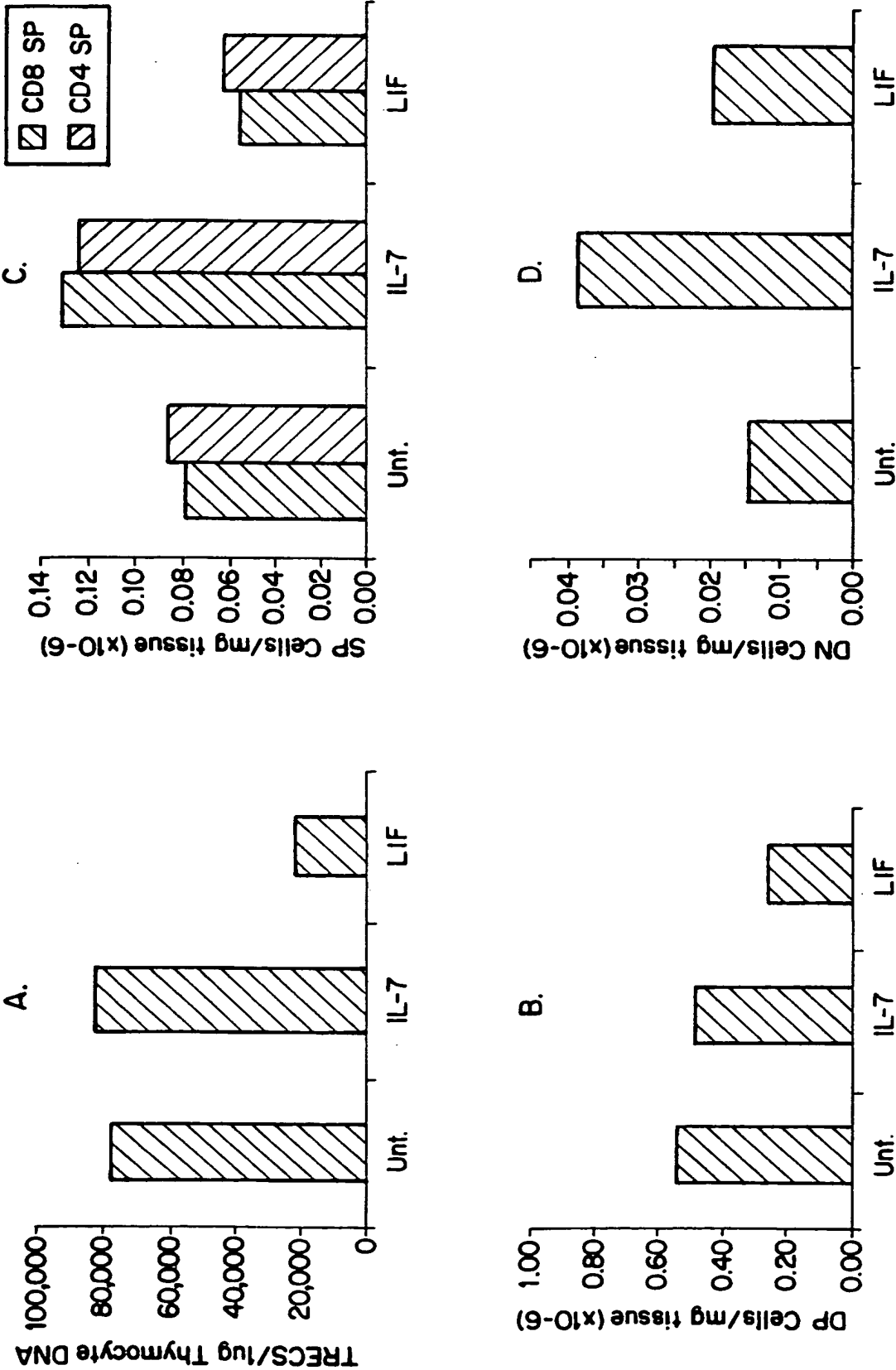
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FIG. 9



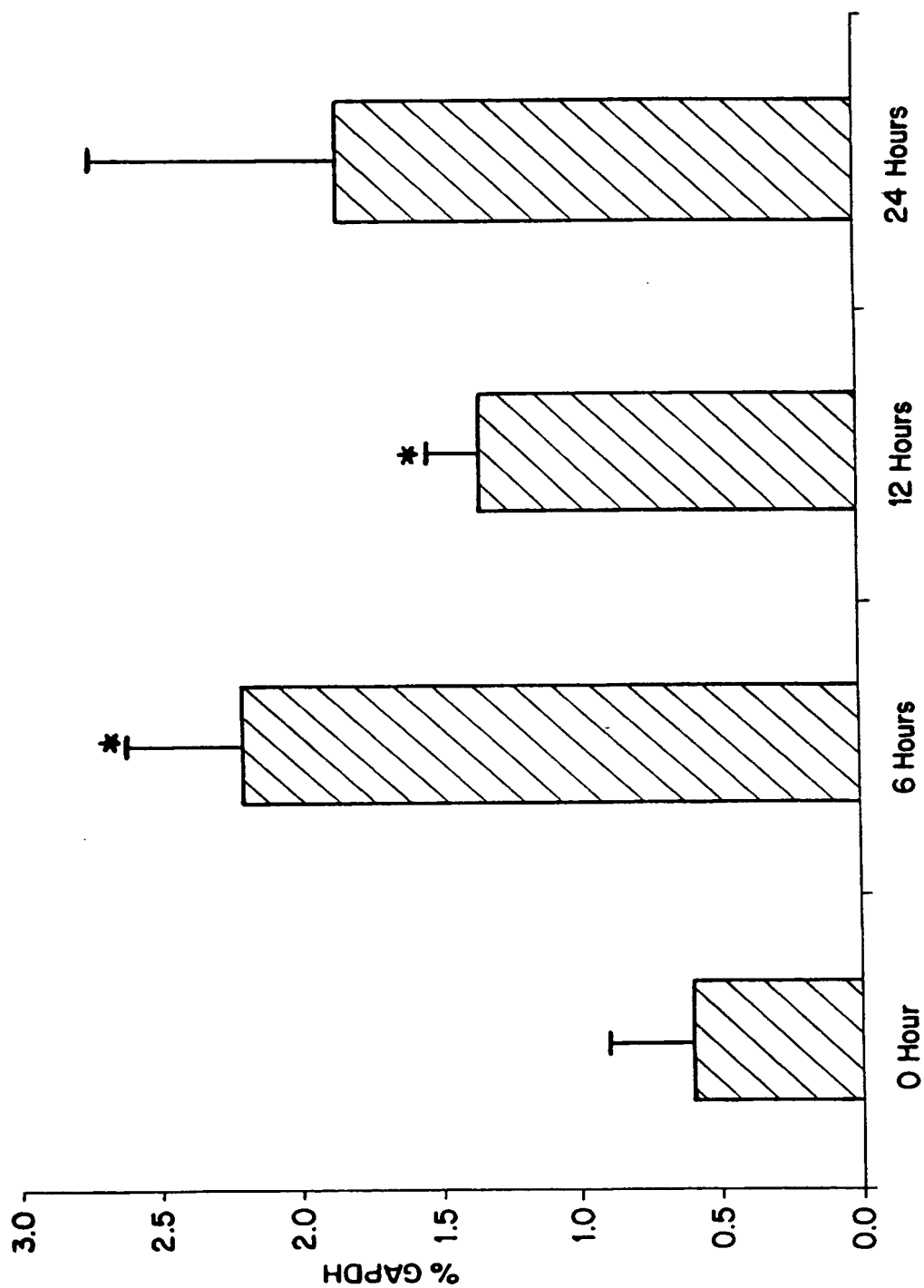
12/14

FIG. 10

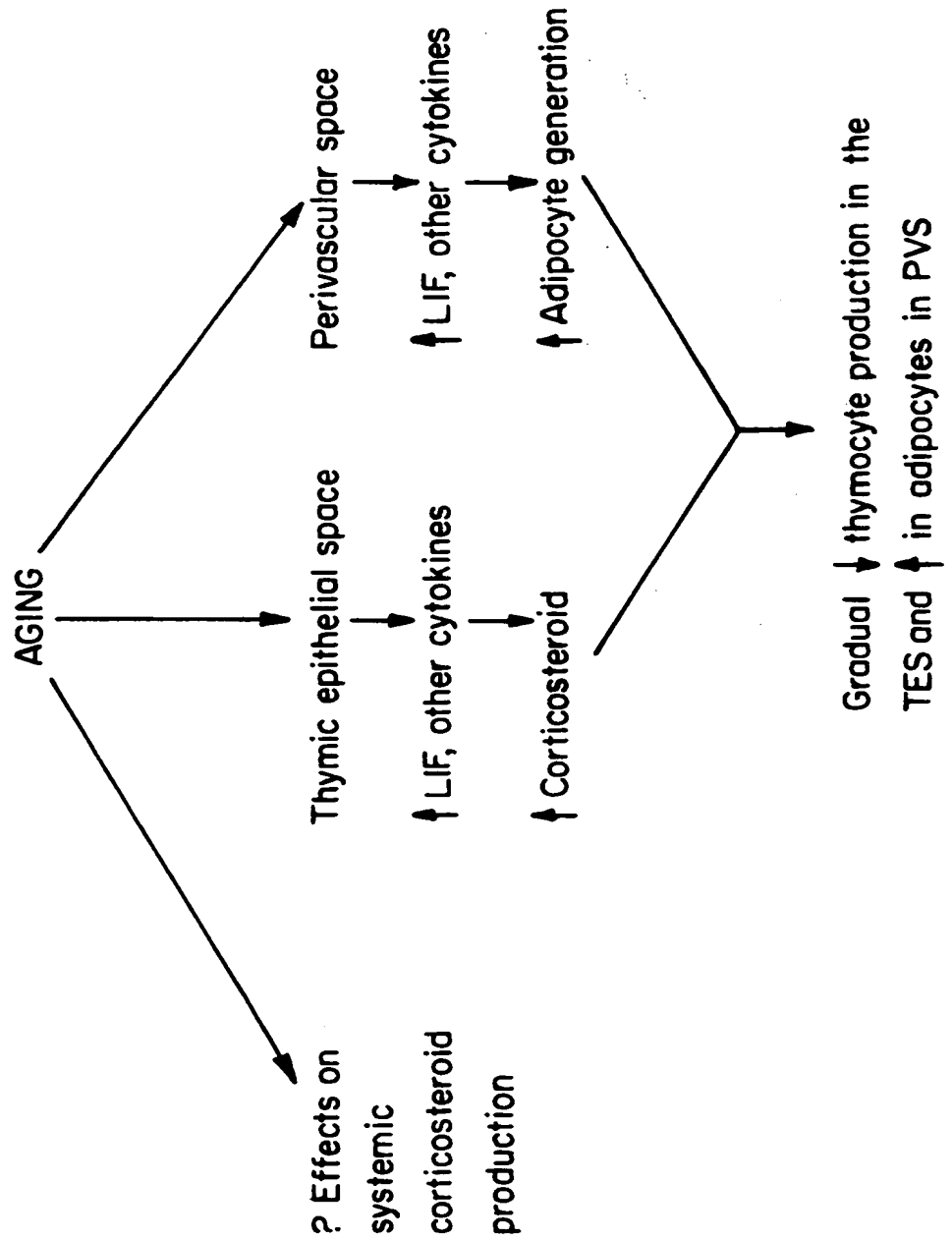


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FIG. 11



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FIG. 12**Thymic Atrophy of Aging**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/10008**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61K/39/19, 38/20, 39/395, 48/00

US CL : 514/2, 8, 12, 885, 44; 424/85.1, 85.2, 130.1, 139.1, 145.1;

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CAS ONLINE, MEDLINE, CAPLUS

search terms: method, therapy, administer, treatment, antagonist, cytokine, agonist, antibody, antisense RNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,683,988 A (CHUNG) 04 November 1997 (04.11.97), see entire document.	1, 4, 5, 7
A	US 5,703,206 A (WOLPE et al.) 30 December 1997 (30.12.97), see entire document.	1-3, 5, 6



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search

21 JULY 2000

Date of mailing of the international search report

04 AUG 2000

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see PCT Gazette No. 04/2001 of 25 January 2001, Section II
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A METHOD OF REVERSING THYMIC ATROPHY

(57) Abstract: The present invention relates, in general, to cytokines and, in particular, to a method of reconstituting the T cell arm of a mammalian immune system in those diseases or conditions in which the T cell pool is damaged. More specifically, the present invention relates to a method of reversing thymic atrophy associated aging and to a method of treating T cell lymphopenia. The methods comprise administering cytokines, agonists thereof, that are underproduced in these physiological states and/or administering antagonists of cytokines that are overproduced.

WO 00/62805 A1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/10008

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/19, 38/20, 39/395, 48/00

US CL : 514/2, 8, 12, 885, 44: 424/85.1, 85.2, 130.1, 139.1, 145.1;

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8, 12, 885, 44: 424/85.1, 85.2, 130.1, 139.1, 145.1;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CAS ONLINE, MEDLINE, CAPLUS

search terms: method, therapy, administer, treatment, antagonist, cytokine, agonist, antibody, antisense RNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,683,988 A (H. CHUNG) 04 November 1997 (04/11/97), see entire document.	1, 4, 5, 7
A	US 5,703,206 A (WOLPE ET AL) 30 December 1997 (30/12/97), see entire document.	1-3, 5, 6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 21 JULY 2000	Date of mailing of the international search report 14 NOV 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer <i>my means</i> PREMA MERTZ Telephone No. (703) 308-0196